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# The interface of mitochondrial DNA transcription and replication

**AKADEMISK AVHANDLING**

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av

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# ABSTRACT

Mitochondria are a dynamic network of subcellular organelles that produce the majority of cellular ATP through the process of oxidative phosphorylation (OXPHOS). The components of the respiratory chain are encoded by two separate genomes, nuclear DNA and mitochondrial DNA (mtDNA), and the proper maintenance of both of these genomic entities is therefore crucial for cellular ATP levels and the survival of the cell. Dysfunction of the respiratory chain leads to cellular energy deficiency and mitochondrial disease, which can manifest in a variety of ways but primarily affects tissues of higher energy demand. Although mtDNA replication and transcription are of vital importance for the cell, the molecular mechanisms behind these processes are not fully understood.

In mammalian cells, mtDNA replication initiates from two major sites, the origins of heavy and light strand replication (OriH and OriL, respectively). Activation of both origins requires a short RNA primer that is generated by the mitochondrial transcription machinery. In this way, mtDNA replication and transcription are intricately linked. At OriH, primer 3' end formation has been suggested to rely on nucleolytic processing of full-length transcripts, but only trace amounts of the nuclease implied in this process are found in mitochondria, making this an unlikely model. In this thesis, we demonstrate that the formation of the primer 3' end is a sequence-dependent event that is directed by the Conserved Sequence Block II (CSBII) sequence element in mtDNA. During transcription of CSBII, the nascent RNA adopts a G-quadruplex structure that causes premature termination of transcription *in vitro*. After transcription termination, the primer RNA remains stably associated with the DNA in a persistent RNA-DNA hybrid called an R-loop. We find that this interaction is mediated by hybrid G-quadruplex structures that form between the RNA primer and the DNA non-template strand. When G-quadruplex formation in either the RNA transcript or in the DNA is prevented, the stable association of the primer RNA is lost.

The mitochondrial RNA polymerase (POLRMT) is also involved in generating the primer at the origin of light strand replication (OriL). In order to define the essential sequence requirements of mammalian mitochondrial OriL, we employ an *in vivo* saturation mutagenesis approach combined with biochemical analysis. Our results support an essential role of OriL in the mouse, consistent with the strand-displacement model of mtDNA replication. Furthermore, bioinformatic analysis demonstrates conservation of the OriL structure in vertebrates.

POLRMT requires two accessory factors for transcription initiation at mitochondrial promoters. However, the necessity of the mitochondrial transcription factor A (TFAM) in this process has been questioned. We use our defined mitochondrial *in vitro* transcription system to confirm the important role TFAM in transcription initiation. The requirement for TFAM can be circumvented by conditions that promote DNA breathing, such as low salt concentrations or the use of negatively supercoiled template. We demonstrate that TFAM has the capacity to generate negative supercoils, which we speculate may contribute to melting of the promoter.